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# Intravascular and Total Body Platelet Equilibrium in Healthy Volunteers and in Thrombocytopenic Patients Transfused With Single Donor Platelets

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**Instrument platelet counts used in corrected count increment (CCI) and percent platelet recovery (PPR) formulas presume the transfused platelets are in equilibrium during the first hour after platelet transfusion. The timing of the pre-transfusion count affects CCI results, and we postulate that timing of CCI post transfusion affects CCI results. Platelet equilibrium using indium-111 platelet transfusions has not been reported. Platelet redistribution was studied in 16 healthy volunteers and 12 thrombocytopenic patients by generally infusing less than 72-hr stored single-donor platelets along with an aliquot of indium-111-labeled platelets by intravenous push. Counts were measured at 10, 15, 20, 60, and 120 min, and 24, 48, 72 hr along with continuous body scanning for 2 hr in healthy volunteers, and static organ scanning in patients and volunteers. Results indicated transfused platelets do not reach intravascular equilibrium for 60 min post-infusion and that the 10-min count cannot detect platelet refractoriness. However, total body equilibrium varies considerably between normal volunteers and thrombocytopenic patients. It is recommended to continue with the 1-hr post transfusion count. Am. J. Hematol. 58:165–176, 1998.** 

**Key words: platelet transfusions; platelet survival; platelet labeling; platelet administration**

## INTRODUCTION

Laboratory tests for evaluating and monitoring platelet therapy are equally as important as monitoring red cell, plasma, or cryoprecipitate transfusions [1]. Platelet counts prior to and after platelet transfusions have been the practice for monitoring platelet transfusions [2]; however, radiolabeled platelet recovery and survival, and bleeding times have also been used [3].

Three formulas use platelet counts for predicting and assessing platelet transfusions. They are the predicted platelet count increment (PPCI) [4,5], the corrected count increment (CCI) [6], and the percent recovery [7] based on platelet counts pre- and post-transfusion, blood volume, dose of platelets, and a splenic pooling factor [4–7]. There are several problems existing with all formulas. A problem inherent in the CCI and percent recovery formulas is the timing of pre- and post-transfusion platelet counts. Both are significantly affected by the pre-transfusion count. Weiner and Kao have observed that the platelet count is reduced at an average rate of $1.0 \pm 0.3 \times 10^9$/L/hr in non-refractory patients [8]. Platelet counts that were determined several hours prior to transfusion may be higher than counts taken immediately before transfusion. Therefore, the CCI may be falsely low, which inappropriately classifies a patient as refractory to platelet transfusions [8].

The post-transfusion count timing may also cause false results. The traditional time has been 1 and 24 hr post-

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transfusion [2,5,10,11], although one study reported 10-min post-infusion counts can replace the 1-hr count and give accurate CCI information [9]. In fact, one author recommended a 10-min count replace the 60-min count [12], whereas most authors suggest a 10- to 60-min count [1–8,10,11]. We tentatively showed the CCI was inordinately high if the platelets are transfused at the time the bone marrow begins regenerating [11]. Also, a problem exists if the platelet count is done close to the platelet transfusion in that no distinction can be made between immune and nonimmune platelet refractoriness [11]. It is, therefore, postulated that the CCI cannot distinguish immune from nonimmune refractoriness if the platelet count is done too close to the platelet transfusion, but this remains to be demonstrated.

O'Connell's study on the 10-min count showed a close linear relationship ($r = 0.98$) between the 10- and 60-min count [9]. This indicated to them that a rapid equilibrium of transfused platelets occurred within a few minutes post-transfusion. Their proposal was to utilize a 10-min count for convenience rather than the 60-min count. Since that study, the post-transfusion count is referred to as the 10- to 60-min time representing physiologic platelet equilibrium in the circulation [5,10]. Nevertheless, the 10-min count has never been shown scientifically to represent platelet equilibrium by using a gold standard such as radiolabeled platelets, or that the 10-min count is equivalent to the 1-hr count.

One previous study correlated percent recovery and CCI with radiolabeled platelet recovery [13], but no previous study evaluated organ distribution and fate of stored transfused platelets by organ scanning. The purposes of this study are to study post-transfusion platelet equilibrium in thrombocytopenic patients compared to healthy controls; to study platelet refractoriness using indium-111 radioisotope platelet recovery and scanning; and, to assess platelet transfusion monitoring methods.

## MATERIALS AND METHODS

### Normal Volunteers

Normal healthy individuals were recruited in a volunteer program. Volunteer recruitment was approved and audited by the Investigational Review Boards for Harbor-UCLA Medical Center and Washington State University. The number of healthy volunteers entered into the study as a control group was 20. The mean platelet survival for the 20 volunteers using linear and multiple hit calculations was $8.7 \pm 0.1$ days and for the exponential calculation it was $7.2 \pm 0.12$ days. This labeling method was done on fresh aliquots of platelets separated from 50 mL of whole blood. These results indicated our labeling technique and data analysis compared to previously published methods [14]. This protocol consisted of labeling an aliquot of fresh platelets prepared from 50 mL of autologous whole blood. Samples for survival studies were collected daily for a minimum of 7 days. Sixteen of these same individuals were studied a second time (test group) using their own platelets collected by apheresis, and stored within 3 days before reinfusing. These 16 individuals served as controls for the patient study. Four left the study due to job or Resident rotation changes. Platelet antibody, platelet labeling, and platelet storage and infusion were treated the same in both groups. The antibody testing, although not perfect, in the control group attempted to assure no autoantibody affected the results.

### Patients

Patients who required platelet transfusions for chemotherapy-induced thrombocytopenia associated with bone marrow hypoplasia were studied at Harbor-UCLA Medical Center (Torrance, CA) and Spokane, Washington, area hospitals. Patients were approved and audited by the Investigational Review Boards for Harbor-UCLA Medical Center and Washington State University.

Our patient studies consisted of four groups in whom platelets were transfused. The first group was incompatible platelets to immune refractory patients, designated immune destruction or an immune refractory group. The second group was immune refractory, but received crossmatch compatible platelets, which is designated immune compatible. The third group received platelets from unrelated and uncrossmatched donors. These patients had non-immune conditions known to shorten platelet survival. This group was designated non-immune destruction or non-immune refractory. The fourth group received unrelated, uncrossmatched platelets that were clinically stable. This group of patients had no immune or non-immune conditions capable of shortening platelet survival. The number of patients studied in each group was: immune refractory ($N = 3$), immune compatible ($N = 3$), non-immune refractory ($N = 4$), and stable hypoplastic ($N = 2$) for a total of 12 patients. All but two patients received platelets stored less than 3 days. Only one transfusion study was done per patient. These are small numbers only because it is the number of patients that completed all of the counting studies, and due to scheduling problems only a third completed the static scanning studies.

### Definitions

Each patient was defined as refractory to transfused single donor, unrelated platelets when the CCI was $<$ 10,000 platelets per $\mu$L per $10^{11}$ platelets transfused per meter squared, and /or PPR or percent radiolabeled platelet recovery was $\leqslant$ 30% at 24 hr, or indium-111 platelet survival was less than 1 day (Multiple Hit calculations) on two consecutive platelet transfusions administered within 1 week. Immune refractoriness was considered

when the above definition occurred with evidence of a platelet associated-antibody. Non-immune refractoriness was considered when the patient met the general definition for refractoriness with no evidence of platelet-associated antibodies and had the associated clinical findings of bleeding, fever, infection, DIC, TTP/HUS, splenomegaly, amphotericin therapy, and previous bone marrow transplant. These clinical factors are defined: bleeding as any physical sign of blood loss and/or a drop in hemoglobin by 1 g within 36 hr; fever as an oral temperature greater than 38°C prior to transfusion; infection as any positive culture of a pathological microorganism from any body source 3 days prior to transfusion; DIC as an elevated D-dimer titer and fibrin degradation product levels concomitant with a prolonged activated partial thromboplastin time; TTP/HUS as a platelet count less than 50,000 per μL with a progressively elevated LDH and microangiopathic hemolytic anemia; and, splenomegaly as a palpable spleen.

### Platelet Antibodies and Pretransfusion Evaluations

Platelet-associated antibodies were detected by a solid-phase red cell adherence system to screen for antibodies using Capture-P or Modified Capture-P screening (Immucor Inc., Norcross, GA). One drop of low ionic strength saline (LISS) and one drop of patient or control serum was added to each well containing screening platelets. After incubation at 37°C for 45 min, the plate was washed four times with 0.9 percent saline after which one drop of anti-human IgG-coated red cell indicator cells was added. The plate was centrifuged at 850*g* for 11 sec and the reactions interpreted visually. A compact red cell button at the bottom of the well was considered a negative reaction. A positive serum caused homogeneous adherence of red cells to the wells. In addition, screening for HLA antibodies was done by using a panel of 140 cells and standard lymphocytotoxicity methods. Positivity was defined if patient serum caused at least 60% cytotoxicity in one or more cells or at least 40% cytotoxicity occurred in two or more cells in the panel. Percent panel reactivity was more than 40% in all patients.

### Platelet Collection, Storage, and Quality Control

Platelets were collected from each volunteer who met AABB and FDA criteria for donor selection by history, examination, and disease testing. Platelet collection was done on the CS-3000 with the original chamber (Fenwal Laboratories, Inc., Deerfield, IL) because of the length of the study and because we did not want to introduce a variable in the middle of the study. However, all platelets were leukocyte reduced by filtration using the Pall PL50 to filter the aliquot of platelets for indium labeling and the PL100 for platelet infusion. All platelet products had less than $1 \times 10^6$ leukocytes with less than 20% platelet losses. Platelets were collected on the CS-3000 using the standard automated platelet collection computer program. Whole blood was processed at 50 mL per minute using an acid-citrate-dextrose (ACD) solution at a ratio of 1 to 11. Each donor or volunteer participated an average of 90 ± 10 minutes in the procedure. The mean total platelet collection was $5.0 \times 10^{11}$ platelets. Platelets were collected at Harbor-UCLA Medical Center ( Torrance, CA) for the first half of the study and at the Inland Northwest Blood Center (Spokane, WA) for completion of the study.

Platelets were stored at 22°C in double thrombocytapheresis PL 732 Fenwal bags (Fenwal Laboratories, Inc.). Platelets had constant horizontal rotation during storage at 70c/m on a Linear Platelet Reciprocator (LPR-1), made by Melco Engineering Corp., Glendale, CA. Each Plateletpheresis product in this study was quality controlled by measuring pH, hypotonic shock, and platelet aggregation. Platelet aggregation was measured on a Chrono-Log platelet aggregometer (Chrono-Log Corp., Havertown, PA) using single agonists, ADP (final concentration of 10 μM), epinephrine (50 μM), collagen (40 μ/mL) (Sigma, Inc., St. Louis, MO) and combined agonists (collagen followed by ADP, and epinephrine followed by ADP). Hypotonic shock was performed according to the procedure described by Valerie et al. [15]. All products had a pH above 7.0, an expected percent hypotonic reversal (71 ± 12.5%), and expected aggregation (>70%) at 24 hr of storage. Aggregation was also done on a sample of the radiolabled platelets, which showed greater than 70% aggregation responses with each of the three agonists.

### Indium-111-Platelet Labeling

This study followed the method recommended by the International Committee for Standardization of Hematology (1988) with modifications [16]. In our preparation of platelets we used a sterile modified calcium-free Tyrode's medium with 0.3 μg of prostaglandin E (PGE), which we call modified Tyrode's (MT) buffer.

In preparing the sample for labeling, an aliquot was obtained from stored platelet concentrates by aspirating 30 mL of platelet concentrate using a 18-gauge needle through a sampling site coupler previously sterilized and inserted into one of the administration ports of the platelet bag. The 30 mL was filtered through a leukocyte-depletion platelet filter (PL-50, Pall Biomedical Inc., Glencove, NY) to reduce leukocytes. Between 10 and 20% of the platelets were retained in the filter. Five milliliters of this sample was lost in the leukodepletion process and 5 mL was used for platelet quality control, leaving 20 mL for labeling. To the 20 mL of platelet concentrate (platelet-rich plasma, PRP), 10 mL of the MT Buffer was added. The platelets were centrifuged at 640*g* for 10 min to pellet the platelets. The supernatant was

saved (tube A). The pelleted platelets were washed in the pelleted state, three times with warm (37°C) MT buffer. After the last wash, 2.5 mL of warm buffer was added and platelets gently resuspended. Indium-111-oxine, 200–300 μCi (14.8–18.5 MBq) (Amersham Corp., Arlington Heights, IL), was added to the platelet suspension. The mixture was incubated for 1 min at 37°C. Volunteers received between 130 and 170 μCi. For the purposes of scanning volunteers received 200 μCi of indium-111.

Our labeling efficiency was 85 ± 9%. The purity of labeling was determined by placing an aliquot of the radiolabeled suspension on Ficoll-hypaque, centrifuging, and performing radioautography to detect red cell and leukocyte contamination. Red cell and/or leukocyte contamination showed the platelet layer as well as one or two other layers representing cellular contamination in the original labeling sample. A pure platelet layer was found using this technique in each of our labeling samples.

### Platelet Administration

Each volunteer only received their own platelets. Platelets were infused by placing an 18-gauge butterfly needle in the anticubital vein. A three-way stopcock was attached to a butterfly, and to a 22-inch Y-platelet transfer set with a check-valve and in line filter for use with a 50-cc syringe for rapid platelet infusion of the platelet concentrate (Product 81251, Medsep, Inc., Covina, CA). A PL-100 leukocyte reduction filter was placed between the platelet concentrate and the stopcock. Therefore, both the concentrate and radiolabled aliquot were leukoreduced albeit via different filters ( PL-50 for aliquot vs. PL-100 for concentrate). The concentrate was infused within 5 min. However, at the point that half of the concentrate was infused, the aliquot of radiolabeled platelets was infused through the stopcock via a syringe and the remaining concentrate infused.

### Blood Volume Calculation

Blood volume was calculated using 68.9 ml/kg times body weight in kilograms for both sexes, which is based on previously published studies [17]. This value in our own study of volunteers also best fit our study population by using regression analysis, of body surface area and weight as previously described. This value was used in all formulas pertaining to blood volumes. We also validated this number by measuring plasma volumes in five volunteers using human serum albumin labeled with $I^{125}$. A nomogram was used to calculate body surface area for the corrected count increment.

### Platelet Transfusion Monitoring

Samples of blood were collected in EDTA for electronic platelet counting on either an ELT-15 (Becton Dickinson, Sparks, MD) or Baker 8000 (Previously Serno, Inc., now BioChem ImmunoSystems, Inc., Allentown, PA) along with a sample for radioactive counting. Samples were collected at 10, 15, 20, 60, and 120 min and 24, 48, and 72 hr. We were most interested in these time intervals to evaluate platelet recovery, because platelets are transfused in patients within 3 days, and because we wanted to evaluate the 10–120-min interval in detail.

The formulas used in this study to compare methods of monitoring platelet transfusions include:
Corrected Count Increment (CCI):

$$\mathrm{CCI} = \frac{\text{Post-minus Pre-Transfusion Counts} \times \text{Body Surface Area } (M^2)}{\text{Number of Platelets Transfused (in } 10^{11})}.$$

The CCI must be greater than 10,000 platelets per μL per $10^{11}$ platelets transfused per meter squared [18].
Percent Platelet Recovery which is:

$$\mathrm{PPR} = \frac{\text{Post-minus Pre-Transfusion Counts} \times \text{Blood Volume (in } \mu L)}{\text{Number of Platelets Transfused (in } 10^{11}) \times \text{0.67 Splenic Factor}} \times 100.$$

Radiolabeled Platelet Recovery, Survival, and Scanning:
Percent survival (recovery) was calculated using the following formula:

$$\text{Percent Indium-111 Recovery}_{72\ \mathrm{hr}} = \frac{\text{Counts/min/ml sample} \times \text{Estimated Blood Volume}}{\text{Counts/min/ml Standard} \times \text{Dilution factor} \times \text{volume of injectate}} \times 100.$$

Percent recovery is previously defined as the percentage of transfused radiolabeled platelets that circulate after infusion and is referenced to measurements within the first 24 hr [14]. For purposes of this study we extend this measurement to 72 hr and gave it the term ''Percent Indium-111 $\text{Recovery}_{72\ \mathrm{hr}}$'' so as to not confuse this radioactive measurement with the platelet counting method described above as percent recovery.

Percent organ radioactivity was determined by dynamic and static imaging studies according to the methods of Heyns et al. [19]. Dynamic scans consisted of continuous scanning for 1 to 2 hr. Static scans were done at 1 and 24 hr and at 5 days. One-third of the volunteers were scanned. Whole body scans were performed using a General Electric Gamma Camera with a StarCam 3000 computer assisted data collection system. We recorded the image on an X-ray film because this method docu-

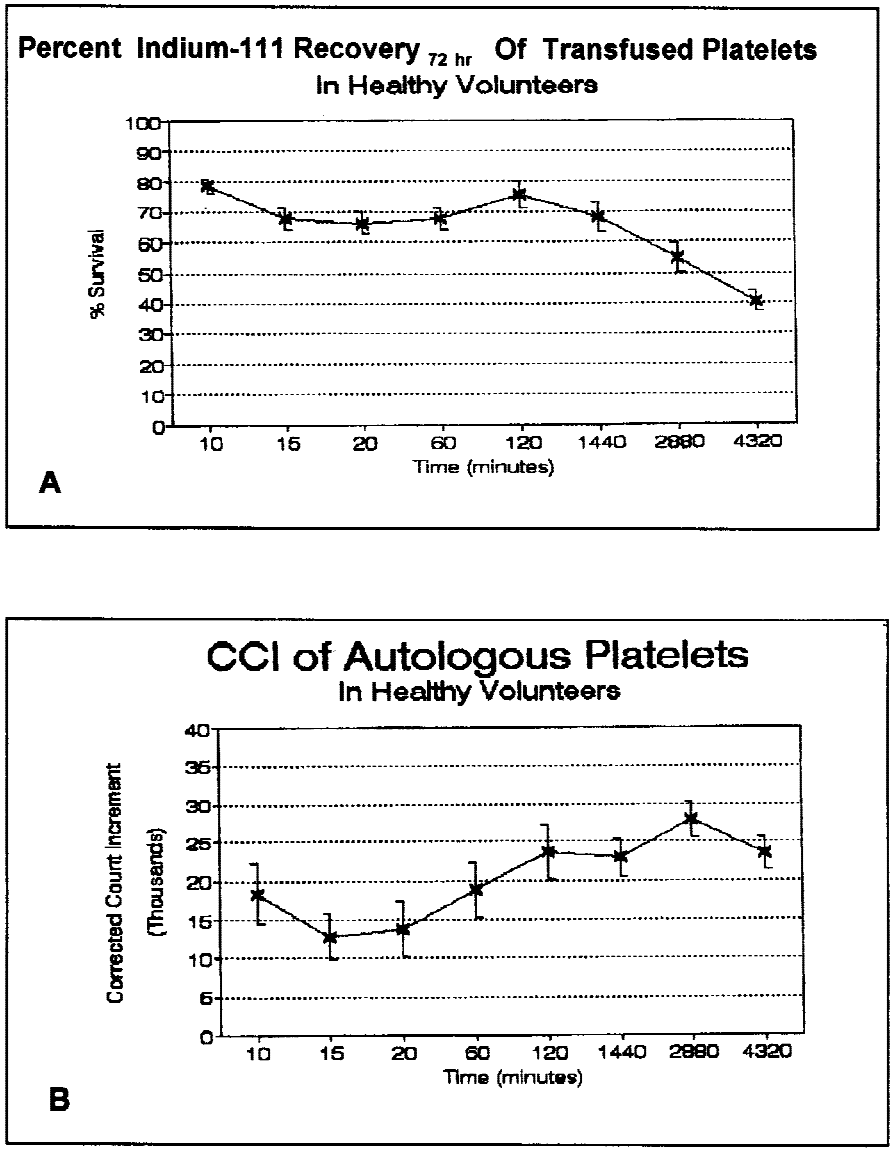


**Fig. 1. Percent Indium-111 recovery$_{72\ hr}$ of transfused platelets in patients (A) and corrected count increment (B) in 16 healthy volunteers transfused with their own platelets.**

ments the highest quality image. The image analysis was done by using select regions of interest (R0I): heart, lungs, spleen, and liver. Percent radioactivity was calculated as:

$$\frac{\text{Anterior Mean counts of the Organ}}{\text{Anterior Mean Whole Body Counts}}.$$

Radiolabeled platelet survival was determined from our data using the linear, exponential, and multiple hit models generated by a computer program (kindly provided by Dr. T.S. Kickler, Johns Hopkins University School of Medicine), taking into account that survival would be inaccurate with only three-time points [20].

### Statistical Analysis

Data was analyzed and descriptive statistical analysis was done by using SSPS-version 6.1.3 [21]. Analysis of variance (ANOVA) was done for appropriate data analysis using SSPS.

## RESULTS

### Controls

Actual CCIs between 14,000 and 16,000 were equivalent to actual PPRs between 50 and 60% as previously calculated. Formulas that use instrument platelet counts, CCI and PPR, had more variance than Percent Indium-111 Recovery$_{72\ hr}$ (Fig. 1). Values above 24,000 for CCI and more than 100% for PPR indicated normal platelet generation, because both values are in the low normal platelet count range. This is shown in Figure 1b using CCI. It is unclear why Percent Indium-111 Recovery$_{72\ hr}$ and CCI decrease between 10 and 60 min in healthy volunteers generating normal platelet counts (Fig. 1).

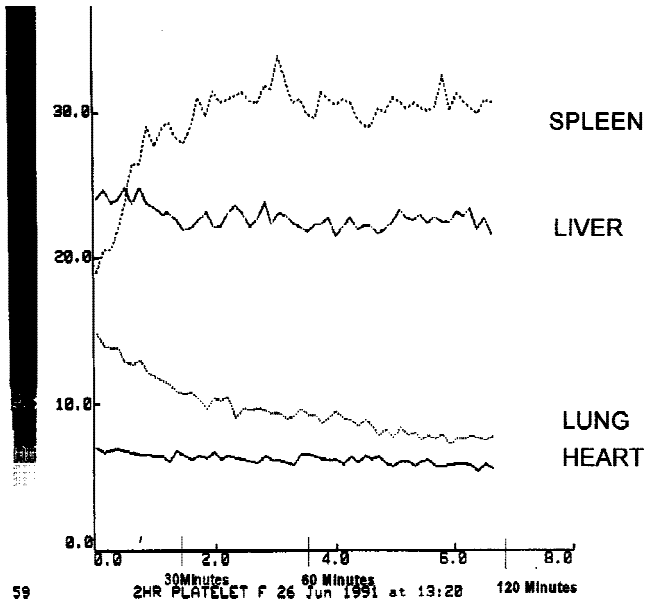


**Fig. 2. Typical graph of continuous organ scanning in a healthy volunteer during the first 2 hr after an autologous 24-hr stored platelet transfusion.**

To determine what time point is best for monitoring platelet transfusions between 10 and 120 min, we did radioactive scanning and performed ANOVA statistical analysis at each time point for normals and for each group with each method: CCI, PPR, and Percent Indium-111 Recovery$_{72\ hr}$. Statistical analysis indicated that there was no time point significantly better than another for normals and for each patient group except for immediate destruction (all immune destruction patients and one non-immune destruction patient). For example, statistical analysis indicated the 10-min time point was no different from the 60- or 120-min time points in immune compatible, stable hypoplastics, and non-immune refractory groups (see Fig. 5A–C). Figure 2 also shows that most of the platelets are in the circulation and the microcirculation (i.e., lungs) in the first 10 min. Percent Indium-111 Recovery$_{72\ hr}$ indicated intravascular equilibrium occurred during the first 2 hr post-platelet transfusion. However, 2-hr continuous total body dynamic in vivo time-radioactivity scanning demonstrated total body (or total organs) platelet equilibrium was reached after 60 min (Fig. 2). The spleen contained 20% radioactivity in the first 10 min after infusion, which increased and maintained 35–45% after 60 min (Figs. 2 and 3). The lungs had 10–15% activity in the first 10 min, which diminished to 4–8% after 30 min. Similarly, the activity in the liver at 10 min was more than 25% and fell to 8–20% after 30 min (Figs. 2 and 3). Therefore, total body equilibrium was reached by 2 hr. Percent organ radioactivity at 1 and 4 hr and 5 days showed a decline (Fig. 3).

## Patients

Table I lists all of the patients studied. Patient categories are based on clinical and platelet- antibody observations: numbers 1 and 2 are stable hypoplastics; numbers 3 through 6 are non-immune destruction/refractory; numbers 7 through 9 are immune destruction/refractory; and numbers 10 through 12 are immue compatible or they have immune destruction, but were transfused with HLA/platelet crossmatch compatible platelets. All products had a pH above 7.0, an expected percent hypotonic reversal (Table I), and over 70% aggregation. Aggregation was also done on a sample of the radiolabled platelets, which showed a normal response. The mean multiple hit platelet survivals for each group of patients are: stable hypoplastics—3.5 ± 2.0 days; immune refractory—0.85 ± 0.015 days; non-immune refractory—1.6 ± 0.37 days; and immune compatible—3.2 ± 1.5 days.

To decide whether one method of monitoring platelets was more accurate and/or precise than another, and whether there was any correlation between methods, e.g., percent radiolabeled survival (% survival) with percent recovery, we evaluated the coefficient of variation for all methods. All three monitoring methods showed the greatest variation in the first 2 hr of measurement. Figure 4 shows this with specific patient groups.

All six patients with platelet-associated antibodies had antibodies with HLA specificity since all patients had positive lymphocytotoxicity. There were no positive Capture-P patients without lymphocytotoxic panel cell positivity. Immune destruction is the only group that is definitely distinguished from the other patient groups (Figs. 4B, 5), but the great variability in the non-immune destruction group can overlap in the immune destruction group. The means for corrected count increment and percent survival (Figs. 4, 5) suggests distinct differences between patient groups. More patients per group are needed for ANOVA, but ANOVA does indicate a trend in distinguishing the different patient groups at 60 min (Fig. 5) using CCI or Percent Indium-111 Recovery$_{72\ hr}$ ($F \geqslant 17$, $P \leqslant 0.07$). Patient platelet survival studies demonstrate a tendency to make a better distinction based on how long the platelets are in the circulation (Table I, survival in days for each group). The group with the greatest variability was the patients with non-immune destruction; Percent Indium-111 Recovery$_{72\ hr}$ had as much variation as CCI (Fig. 4C,D). This concludes three important issues: (1) most of the variability in any monitoring method is within the first 2 hr post-infusion; (2) as the pathophysiologic mechanism of transfused platelet destruction becomes more heterogeneous (e.g., Fig. 4, nonimmune destruction) variability becomes greatest; and, (3) radiolabeling is no improvement over CCI or any other method for monitoring platelet transfusions despite patient heterogeneity.

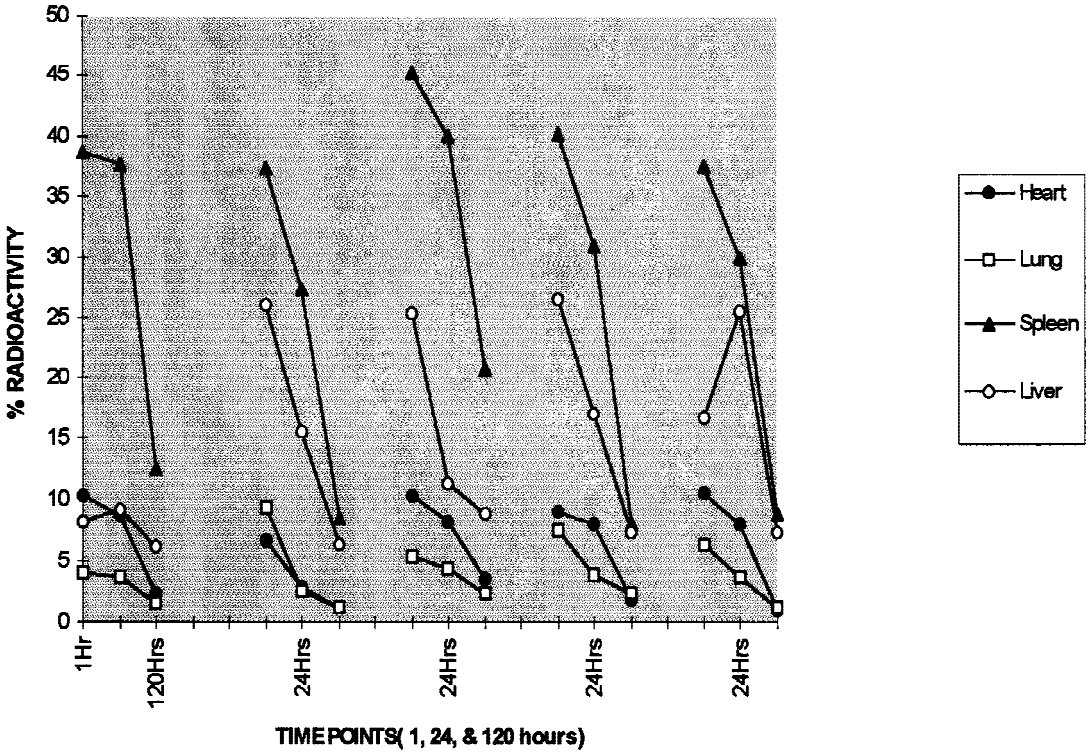


**Fig. 3. Static radioactive organ scanning of five healthy volunteers at three separate time points.**

**TABLE I. Patient Groups Based on Platelet Survival, and Clinical and Laboratory Data***

| Patient number | Primary disease | Storage time of platelets (days) | Hypotonic reversal (%) | Survival (days) | | | Clinical association with refractoriness | Platelet-associated antibody | Refractoriness classification |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Linear | Exponential | Multiple hit | | | |
| 1 | Myelofibrosis, splenectomy | 5 | 47 | 3.16 | 1.51 | 2.08 | None | Negative | Stable |
| 2 | AML | 1 | 67 | 7.06 | 3.5 | 5 | None | Negative | Stable |
| 3 | ALL | 3 | 78 | 3.34 | 1.85 | 1.73 | GI bleed, fever | Negative | Non-immune refractory |
| 4 | ALL | 2 | 67 | 2.17 | 1.62 | 1.68 | Infection, fever | Negative | Non-immune refractory |
| 5 | AML | 3 | ND | 2.28 | 1.24 | 1.09 | Mild bleeding, fever | Negative | Non-immune refractory |
| 6 | CML | 3 | 68 | 3.7 | 2.4 | 1.95 | Splenomegaly | Negative | Non-immune refractory |
| 7 | Blast crisis | 5 | 35 | 2.4 | 0.73 | 0.85 | Fever | Positive | Immune refractory |
| 8 | AML | 1 | 70 | 2.2 | 1.2 | 0.86 | Fever | Positive | Immune refractory |
| 9 | AML | 3 | 84 | 1.6 | 1 | 0.83 | Infection, fever | Positive | Immune refractory |
| 10 | Aplastic Anemia | 2 | 76 | 5.9 | 3.4 | 4.2 | None | Positive | X-match and HLA compatible |
| 11 | Aplastic Anemia | 3 | 51 | 4.7 | 2.5 | 3.9 | None | Positive | X-match compatible |
| 12 | MDS | 2 | ND | 2 | 1 | 1.5 | None | Positive | HLA and X-match compatible |

*Patients 1 and 2 are stable hypoplastics; patients 3–6 are non-immune refractory; patients 7–9 are immune refractory; and patients 10–12 are immune compatible. AML, acute myelogenous leukemia; ALL, acute lymphocytic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; HLA, human lymphocyte antigens.

We observed four patient's percent organ radioactivity at 2 and 24 hr (Fig. 6). Patients A and B had immune destruction, C had splenomegaly, and D had fever and infection. The immune destruction patients had 50% less radioactivity in the spleen and liver than normal volunteers. The other two patients had twice normal radioactivity in these organs.

## DISCUSSION

We designed our study to investigate transfused platelet equilibrium and comparative counting methods in persons generating their own platelets as controls and in thrombocytopenic patients. Equilibrium was studied by taking radiolabeled platelet counts and instrument plate-

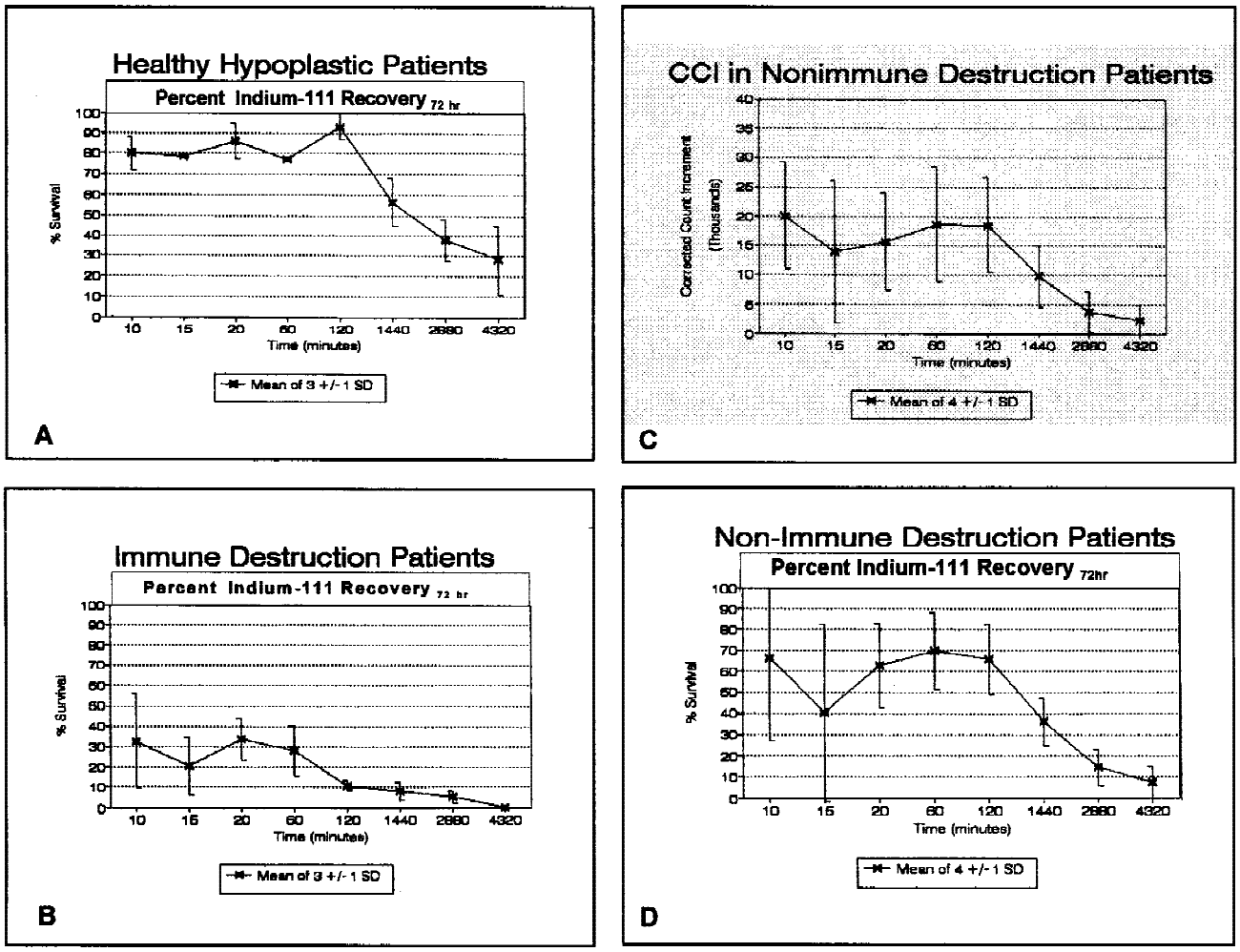


**Fig. 4. Specific patient groups show differences in percent Indium-111 recovery$_{72\ hr}$ between stable hypoplastics (A) vs. immune refractory patients (B). C,D: Large variance in the non-immune refractory patients for CCI and percent Indium-111 recovery$_{72\ hr}$.**

let counts for formulas at 10, 15, 20, 60, and 120 min and continuous organ scanning for 2 hr in five healthy volunteers. Comparing the mean and standard error of the mean (Fig. 1) of radiolabeled percent survival and CCI, the 10- and 60-min counts are nearly identical, but between 10 and 60 min there is a decrease in counts that occurs in healthy volunteers. Why there is a slight decrease in all counting methods between 10 and 120 min is unclear, but it may be explained by organ redistribution during this interval or by a mechanism similar to the destruction of autologous platelets found in posttransfusion purpura where the transfused platelets decrease their own platelets [22]. However, statistical analysis of counts in normals indicates that there is no time point that is significantly different between 10 and 120 min. This suggests platelet equilibrium exists by platelet counting methods. To further investigate equilibrium, we did dynamic in vivo time-radioactivity curves to reflect changes in organ activity over specific time points. Continuous body scanning for radioactive labeled platelets over 120 min indicated that transfused platelets do not reach hemodynamic equilibrium immediately; nor are they immediately sequestered in the spleen (Fig. 2). In the volunteers we scanned, platelets were immediately within the circulation and microcirculation such as the lungs and liver (within 30 min of infusion). Heyns et al. also noted accumulation in the lungs that they attributed to platelet aggregates [19]. We treated our platelets with prostaglandin E to prevent preformed aggregates or spontaneous aggregation in the circulation [23]. Since there was no variance between subjects studied regarding this increased immediate lung activity, this represents the normal platelet kinetics after infusion and not aggregate trapping. The spleen increases activity between 30 and 60 min, and by 2 hours, equilibrium is reached. Our dynamic continuous image recording was similar to those of Heyns et al. [19] and our anterior recording method appeared no different from their geometric re-

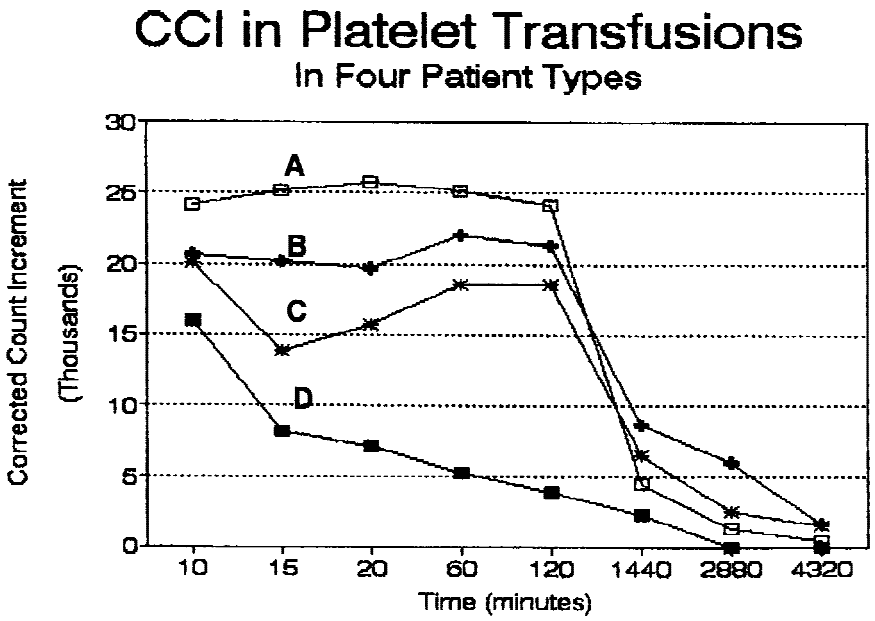


**Fig. 5. The means for corrected count increment for four patient groups: (A) immune compatible; (B) sable hypoplastics; (C) non-immune refractory; (D) immune refractory.**

cording. However, our study had different static percent organ radioactivity results than Heyns et al. [19]. They performed counts on three organs of interest (spleen, liver, and heart), every other day for 10 days. The activity in the spleen and liver gradually increased over the 10 days. In contrast, our measurement of organ activity decreased more than 5 days in our volunteers (Fig. 3). This is possibly explained by the use of stored platelets in our study, whereas Heyns et al. used fresh autologous platelets [19].

The platelet studies by Aster 30 years ago [24] with $^{51}$Cr-labeled platelets revealed the immediate static sequestration of approximately one-third of the transfused platelets in the spleens of healthy volunteers. Since then, there have been few studies investigating the dynamics of the spleen regarding platelet circulation [25,26]. Our study indicates by indium-111 platelets that 20 to 30% of platelets pool in the spleen, but not as immediate (i.e., 5–10 min) as Aster and others have shown [9,24], but rather after 60 min. In fact, there is no static pooling in the initial 30 to 60 min, but rather dynamic redistribution is occurring. Heyns et al. [25] demonstrated with indium-111 in apheresis donors that by removing platelets from the body, roughly one-third came from the spleen and another third from the rest of the body. Both diminished in parallel. This indicates a dynamic equilibrium between the circulation and the spleen. The major sites of sequestration were 37.9 ± 20% in the spleen and 30.2 ± 5.6% in the liver [25]. By analyzing continuous scanning (Fig. 2), 35–40% of the platelets go to the spleen within an hour and 25% go to the liver. And, by utilizing the time-activity curves (Fig. 2), it appears that the platelets predominantly remain linear in these organs for at least 2 hr, but by counting methods (Fig. 1B), the platelets have actually increased in the circulation. This also proves there is dynamic movement of platelets in and out of these organs.

Our results raise issues regarding transfused platelet equilibrium, redistribution, immediate vs. delayed destruction, platelet infusion methods, timing of post-transfusion counts to distinguish immediate from delayed platelet destruction, and the definition of platelet refractoriness.

Heyns et al. refer to the first 2 hr as the equilibrium phase [19], whereas others call this the redistribution phase [27]. Using platelet counting methods, O'Connell et al. [9], Rinder et al. [13], and our own study indicate intravascular equilibrium exists between 10 and 120 min. However, total body or total organ equilibrium does not occur for an hour after platelet infusion. By using quantitative dynamic and static scanning techniques, we found organ or total body redistribution of platelets was occurring during the first hour post-transfusion. Therefore, from evidence in this study we prefer the term intravascular platelet equilibrium within 60 min post-infusion and platelet redistribution after 60 min because total body/organ equilibrium is only reached after platelets redistribute from organ pools (spleen, liver, bone marrow, etc.) into the circulation. After total body redistribution is complete at 2 hr in heathy volunteers and stable thrombocytopenic patients, both the intravascular and organs decrease in counts and organ activity, which represents a second distribution phase for transfused platelets.

The divergent results between platelet counting methods and imaging in healthy volunteers in this study raise the question as to the optimal platelet infusion method and optimal times for post-transfusion counts. The post-

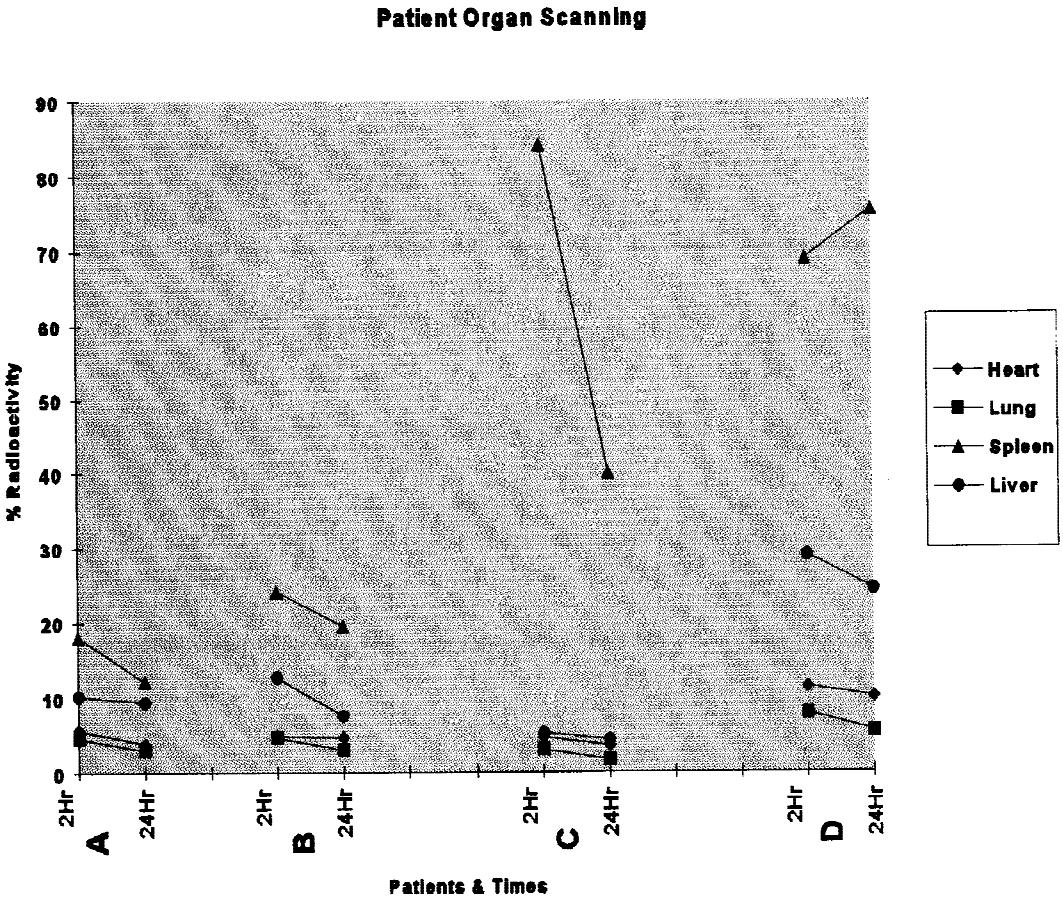


**Fig. 6. Static radioactive organ scanning of four patients at two separate time points. Patients A and B had immune destruction; C had splenomegaly; and D had a fever and infection.**

transfusion counting time depends on two factors: the rate of platelet infusion and what one wants to measure. We used a rapid platelet infusion method (syringe push) so that all of the platelets were transfused within 5 min. O'Connell et al. [9] infused their platelet dose within 30 min, but practically, most intravenous therapists infuse platelets for an hour or more, though it has been advised to transfuse platelets within 10–20 min [28]. Most studies give no indication as to the length of time for their platelet infusions, nor to the length of platelet storage, which also impacts platelet counting methods [29].

Since the first report using CCI and HLA matched platelets in refractory patients [6], refractoriness has been primarily associated with alloimmunization [2,7,10]. Although other etiologies were recognized for causing poor responses [2], we distinguished immune vs. non-immune refractoriness [11]. Refractoriness is often used to indicate a poor response 1 hr post-platelet infusion [2,7,10]. However, we and others defined refractoriness as repeated inadequate 1-hr post-transfusion increments on successive days or successive transfusions [27,30]. One incident with a poor response may result from 5-day stored platelets or ABO mismatch rather than more severe conditions such as alloimmunization, splenomegaly, sepsis, etc., that truly cause poor responses to more than one platelet transfusion [31,32]. The definition of refractoriness is important in understanding the etiology and pathophysiology of various factors that influence platelet increments.

It remains unclear which factors influence platelet increments at 10 min, 1 hr, and 18–24 hr post-platelet transfusion. We postulated that the 10-min count would not be useful in diagnosing platelet refractoriness, but rather the 60- or 120-min count would prove effective. The 10-min count study by O'Connell et al. made no comparisons between the 10- and 60-min counts and the factors that influence platelet increments at either 10 or 60 min [9]. All of their patients fit the categories of stable thrombocytopenic patients, HLA-matched transfused patients, and stable autologous transfused patients. Therefore, their high correlations of the 10-min count with the 1-hr count match the same findings of our two groups identified as immune compatible and stable hypoplastics (Fig. 5A and B). Our study indicates that the 10-min count is of no value in distinguishing the different factors that influence platelet counts.

Daly et al. initially showed the 1-hr count and the 18–24-hr counts could differentiate alloimmunization (CCI < 10,000 at 1 hr) from nonimmune causes, e.g., sepsis, fever, splenomegaly (>10,000 CCI at 1 hr, but <10,000 at 18–24 hr) [2]. Bishop et al. found the 1-hr count could not distinguish between alloimmunization to HLA antibodies and the following nonimmune factors: splenectomy, bone marrow transplantation, disseminated intravascular coagulation, Amphotericin B, and splenomegaly [31]. The same results were subsequently shown with the 20-hr count in that the 20-hr count correlated with the 1-hr count [32]. Therefore, nothing was gained with the 1-hr count over the 20-hr count and the next day count was advantageous for hospitalized patients. However, they did not measure the degree of poor response with associated factors that influence the increment. Through regression analysis they simply determined what factors predicted the greatest impact on the 1- and 20-hr counts. Although factor predictors are useful as a differential in etiologies, definition of shortened survival and recognition of variability in the pathophysiology of each factor is important in distinguishing these factors using methods to determine shortened survival. Some studies use CCIs of 10,000 and others 7,500 to define shortened survival. If we use the guidelines by Slichter of <30% recovery at 1 hr, <20% recovery at 18–24 hr or a survival <2 days for a poor response, we may determine immune from nonimmune refractoriness [10]. Even with the small numbers in this study, by using Slichter's criteria, all of our immune refractory patients had survivals less than 2 days, 1-hr recoveries of 30%, and 24-hr recoveries of 20%. Our nonimmune refractory patients had slightly higher recoveries and survivals. This study, however, did not have patients with prior bone marrow transplants, Amphotericin B therapy, or DIC in the nonimmune group, which may be similar to shortened recovery and survival of the immune refractory group. This study at least suggests that by using strict guidelines or definitions of shortened survival, one may distinguish immune from nonimmune causes of refractoriness at 1 hr.

Statistical analysis has differentiated many factors affecting survival of transfused platelets [27,31,32], but as Friedberg et al. indicate there is tremendous variability in patients [27]. Multiple factors may exist together; each patient may be sensitive to one or a few particular factors; or there is marked intraindividual variation. We observed the most variability in the nonimmune refractory patients. However, using dynamic or static radioisotope scanning over time will prove very useful in understanding the pathophysiology of each factor causing shortened platelet survival of transfused platelets. Two examples from this study are informative. We expected the immune refractory patients to have mostly hepatic and splenic activity at 2 and 24 hr (Fig. 6). Instead, we found comparatively below normal activity in these organs of red cell and platelet immune destruction. This indicates the platelets were mostly destroyed within 2 hr and the mechanism was mostly intravascular. The second example of fever and infection had as much splenic activity as the patient with splenomegaly (Fig. 6). Patients with sepsis and infections develop reactive splenomegaly, which explains this increased splenic activity and probable platelet destruction [33].

The original intent for the 10-min count was convenience for physicians and for patients in the outpatient clinic setting [9]. However, a long infusion time with a 10-min post-infusion count would not diagnose immediate platelet destruction. Convenience for the patient and physicians can also be achieved by intravenous push, followed by a 60-min count. This method will distinguish immediate platelet destruction and it takes the same amount of time for the patient as the slow infusion method followed by a 10-min count. This study lacks the large patient numbers required for statistical analysis, but does indicate poor correlation of the 10-min count with shortened platelet survival. And, we do show the need to utilize dynamic and static radioisotope scanning over several time points to understand the pathophysiology of various factors affecting transfused platelet survival, in particular bone marrow transplant patients. Percent organ radioactivity and dynamic in vivo time-radioactivity curves are very useful, but neglected methods to study platelet redistribution. The only technique to measure platelet redistribution dynamically is by using indium-111-labeled platelet recovery and scanning.

In summary, modern scintillation cameras and computerized data collection systems allow more accurate studies of platelet kinetics. Using counting and scanning, indium-111 as a radionuclide offers the advantage of proving previous postulates regarding platelet sequestration and platelet kinetics, e.g., various organ pooling and platelet mobilization from the lungs and liver, and the exact size of the splenic platelet compartment. To fully understand the physiology of transfused platelets in immune and non-immune platelet refractory patients, dynamic and static total body and organ scanning should accompany indium-111-labeled platelet percent survival and platelet survival studies. By using these methods, we determined the 10-min count is not useful for detecting refractoriness and that the equilibrium phase is not complete until at least 60 min post-platelet infusion.

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